THE ASSOCIATION OF AMERICAN GEOGRAPHERS

The eighth annual meeting of the association was held in Washington, December 28–30, 1911. Through the kindness of the National Geographic Society, the sessions were held in Hubbard Memorial Hall, and luncheon was provided for those in attendance.

The president, Professor Ralph S. Tarr, of Cornell University, presided, and gave the president's address. His subject was "The Glaciers and Glaciation of Alaska." Professor Martin read a memorial of Professor Christopher Webber Hall, who died on May 10, 1911. In addition to these addresses, thirty-six papers were read by members and by several others on invitation. Paper on subjects in meteorology were more numerous than at any previous meeting of the association.

On Friday evening the association met with the Geological Society of America to hear the address of its president, Professor W. M. Davis, upon the subject "The Relations of Geology and Geography." This was followed by a smoker given to both societies at the Cosmos Club by the Geological Society of Washington.

The first volume of the *Annals* of the association is now in press and will appear during the winter under the editorship of Professor R. E. Dodge. Announcement was made of the election of the following officers for the year 1912: *President,* Rollin D. Salisbury; *First Vice-president,* Marius R. Campbell; *Second Vice-president,* Isaiah Bowman; *Secretary,* Albert Perry Brigham; *Treasurer,* Nevin M. Fenneman; *Councillor for three years,* Lawrence Martin. The next annual meeting will be held in New Haven.

Albert Perry Brigham,
Secretary

THE SOCIETY OF AMERICAN BACTERIOLOGISTS

At the Washington meeting of the society from December 27–29, the following papers were presented:

Biochemical Problems in Bacteriology (president's address): F. P. Gorham.

This address will appear in Science.

The Classification of the Streptococci by their Action upon Carbohydrate Media: C.-E. A. Winslow.

When Gordon first suggested the use of carbohydrate media for differentiating streptococci, it seemed that this long-mooted problem was at last likely to be solved. The work of the last five years has, however, left matters in almost as confused a condition as ever, since different observers obtain such varied, and in some cases, conflicting results.

The author attempts to review in this paper the results of four recent American investigations, including 302 fecal streptococci, studied by Winslow and Palmer, 101 milk streptococci studied by Broadhurst, 65 throat cultures studied by Hilliard and 17 cultures from various sources examined by Bellinger, of Meadville College.

In all these cases the amount of acidity produced was determined by titration. The first point brought out is that the dividing line between fermenting and non-fermenting strains lies at about 1.2 per cent. acidity. The English results obtained by Gordon and Houston have been obtained by the use of litmus without titration, and if their solutions were not exactly neutral at the start, they must have classified as fermenting many organisms which really belong to the non-fermenting class. A careful comparison of the English results for particular groups shows, indeed, that they include a much greater percentage of positive records than the American ones.

Experience seems to indicate that systematic studies of the carbohydrate relations of the streptococci should be carried out by titration with phenolphthalein as an indicator. Media may be made up from meat extract (each batch checked by controls inoculated with *B. coli*) and adjusted to an initial reaction between neutral and .5 per cent. acid. Dextrose, lactose, saccharose, salicin, inulin, mannite and raffinose should all be used for diagnosis for the present at least, and titration may be made after cultivation for three days at 37° C.

When studied in this way, cultures producing over 1.2 per cent. acidity more than that initially recorded being considered positive, the media are acted upon (with occasional exception) in accordance with a definite order of fermentability, which is quite different from the order which prevails in the colon group. If only one carbohydrate is attacked that one is usually dextrose. Lactose and saccharose come next and particularly with fecal streptococci. Salicin is almost as commonly acted upon. Mannite is rarely attacked except by streptococci from milk and human feces, while raffinose is rarely attacked except by organisms from bovine feces. Inulin fermenters occur only abun-

dantly in milk. These facts correspond with the chemical relationships of the sugars, the monosaccharide and disaccharide being most readily attacked, next the glucoside, salicin, which breaks up into a simple sugar, while the more complex carbohydrates are less available. The streptococci form a more or less continuous series from those which attack no sugar at all to those which ferment all the carbohydrates tested. Occasionally a strain reacts with a substance lower in the series of fermentability while failing to do so in a sugar usually more available, but this is rare. Characteristic forms appear in the throat and in the feces of man and other animals, so that the method promises to prove of practical as well as theoretical value.

A Comparison of Streptococci from Milk and from the Human Throat: E. C. STOWELL and C. M. HILLIARD.

The present study of throat and milk streptococci was undertaken with the object of establishing a method for distinguishing between organisms isolated from the two sources. (The small number of strains examined (70) renders any present generalizations only tentative.)

The streptococci used were all isolated from fresh specimens in our laboratory by cultural and morphological examinations. Quantitative acid production was studied in six carbohydrate media; dextrose, maltose, lactose, saccharose, raffinose and mannite. The tubes were incubated at 37 and at 20 degrees for 72 hours. The maltose results are neglected in the present discussion.

Below 1.2 per cent. acid production at 37° is considered a negative reaction. All but four cultures on this basis fermented one or more of the carbohydrates; 83 per cent. may be placed in three groups according to the correlation of their capacity to ferment the media used. The following table shows this distribution:

Fermented	Per Cent. Throat Cultures	Per Cent. Milk Cultures
Dextrose only	22.2	6.3
Dextrose and lactose	20.4	38.0
Dextrose, lactose, saccharose ...	33.1	57.7

Grown at 20° the first reaction mode falls at 0.5 per cent. and on this basis the milk cultures fall into the same general groups as shown above, but the throat streptococci show 50 per cent. not to ferment at all, and 40 per cent. to ferment dextrose only.

A comparative study of the cultures isolated from sore, inflamed, or otherwise "abnormal" throats and from normal throats showed no essential differences; the reaction curves follow the same general contours throughout.

(No clue to the relationship of the forms studied was obtained from the morphology or staining reactions.)

Our work leads us to make three preliminary conclusions:

1. Streptococci from the human throat and from milk very generally ferment one or more of the sugars, dextrose, lactose and saccharose, attacking them most readily in the order named. They do not generally ferment raffinose or mannite.

2. The streptococci of the sore and the normal throat show no cultural differentiation in relation to the carbohydrates used. Virulence tests would perhaps have separated the two groups.

3. Milk streptococci are much more facultative than throat strains in relation to the temperature at which they are grown. This is, perhaps, the most valuable information obtained as a differential feature between chained cocci from the two sources.

A Study of Thirty-five Strains of Streptococci Isolated from Samples of Milk: GUSTAV F. RUEDIGER.

The paper points out that *Streptococcus lacticus* can be differentiated from *Streptococcus pyogenes* by means of blood-agar plates. *Streptococcus pyogenes* produces small colonies surrounded by a large zone of hemolysis, whereas *Streptococcus lacticus* produces green or grayish colonies with very little or no hemolysis.

Streptococcus lacticus has no sanitary significance, as it is found in nearly all samples of clean, soured, or fresh milk, and very often in the healthy milk ducts. *Streptococcus pyogenes,* on the other hand, seems to occur but rarely in milk and is indicative of the existence of an inflamed condition of the udder of the cow furnishing the milk.

A Biometrical Study of Milk Streptococci: JEAN BROADHURST.

This comparative study of carbohydrate fermentative reactions is based upon streptococci isolated from milk plates which were made in the routine milk examination by the New York Department of Health. One hundred strains were isolated, and as rapidly as purity was assured transferred to eight of the Gordon test media:

neutral red for reduction, milk for coagulation, and the following carbohydrates for acid formation: saccharose, lactose, salicin, inulin, mannit and raffinose.

Neutral red has since been discarded by Houston as not sufficiently diagnostic. Milk is apparently open to the same objection.

Only the six carbohydrates therefore remain for consideration. Houston used them all (and coniferin) in his milk tests, but secured qualitative results only. Quantitative ones have been recorded for 300 fecal (human, bovine and equine) strains by Winslow and Palmer for dextrose, lactose, raffinose and mannit.

Sugar-free broth was used, and the streptococci therefore began their growth with an initial acidity of 1.3 to 2.2 per cent. (deducted from all recorded results). After 72 hours' growth, titration (with phenolphthalein) showed the following results:

1. Non-fermenting and fermenting groups are found for each of the six carbohydrates tested. The dividing line lies near 1.5 per cent. (higher than among the fecal streptococci).

2. Streptococci ferment these carbohydrates in a large percentage of the strains: lactose, 74 per cent.; saccharose, 68 per cent.; salicin, 77 per cent.; inulin, 37 per cent.; mannite, 26 per cent., and raffinose, 13 per cent.

3. The amount of acid is remarkably large (highest in saccharose, 8.4 per cent.).

4. The reaction combinations observed are numerous, and therefore small: 4 groups of 10 or more strains, and 4 of 5 to 9 strains; these include about 75 per cent. of the strains.

5. These reaction groups are apparently not correlated with morphological characters.

6. The high records for acidity are even more remarkable when the initial acidity is considered.

DISCUSSION OF STREPTOCOCCI

W. L. Holman referred briefly to the Andrade indicator—acid fuchsin decolorized by sodium hydrate—as used in the pathological laboratories in Pittsburgh. He spoke of the differential media for streptococci in use by them, viz., blood agar, lactose, mannit, salicin and inulin broth and laid particular stress on the fermentation or non-fermentation of the various carbohydrates, believing that of greater importance than the exact titration of acidity produced. The use of the hemolytic test he advocated as of great importance in separating two large groups of the streptococci.

A Study of the Diphtheria Group by the Biometric Method: M. E. MORSE.

This study was undertaken for the Boston State Hospital at the instance of Dr. E. E. Southward. The writer is greatly indebted to Professor C.-E. A. Winslow for assistance.

The characteristics which were chosen as the basis of classification were:

A. Morphology. The cultures of diphtheria bacilli were grouped as granular, segmented and those in which solid and small granular forms predominated.

B. Vigor of growth.

C. Chromogenesis.

D. Quantitative determination of acidity in 1 per cent. dextrose, maltose, glycerin, saccharose and dextrin broth.

E. Virulence (guinea-pig inoculation).

F. Toxin production.

G. Immunity reaction (fixation of complement and conglutination). This part of the work is still in progress, and will be reported later.

187 strains of Klebs-Loeffler bacilli, 76 of diphtheroids and 23 of Hoffmann's bacillus have been studied.

RESULTS. I. DIPHTHERIA GROUP

A study of the relationship between morphology and virulence shows that 61 per cent. of the granular cultures were virulent, and only 34 per cent. of the segmented cultures. The solid and small granular cultures were, with one exception, non-virulent.

The group of virulent diphtheria bacilli forms more acid in dextrose, maltose and dextrin broth, than does the non-virulent group.

There are forms sharing the characteristics both of the Klebs-Loeffler group and the diphtheroids.

II. DIPHTHEROID GROUP

The common diphtheroids found on the human body fall into three sub-groups.

Group A.—The so-called "Hoagbacillus." Medium-sized bacillus with solid, barred and wedge forms. On serum, a very heavy glistening salmon-pink growth. Ferments dextrose and saccharose always, maltose and glycerin infrequently; and never dextrin.

Group B.—Morphologically larger and thicker than "Hoagbacillus"; forms with clear-cut bars predominate. On serum, a heavy yellow, dry growth. Ferments dextrose, but not saccharose; usually maltose, glycerin frequently.

Group C.—Differentiated primarily by slow, scanty, colorless or white growth. Morphologically smaller than organisms of group *B;* thick, curved, with solid, barred and wedge forms. Ferments dextrose, always; saccharose usually, maltose and glycerin in 50 per cent. of cultures. The xerosis bacillus apparently belongs to this group.

CONCLUSIONS

1. The division of diphtheria bacilli into virulent and non-virulent varieties is justified by differences in morphology and degree of fermentative powers, and by the absence of intermediate grades of virulence, as shown by animal inoculations.

2. The common "diphtheroid" organisms found in the human body fall into three sub-groups.

3. B. Hoffmann's has no biological relationship either to the diphtheria bacillus or to the diphtheroids.

A Biometric Investigation of Certain Non-spore-forming Intestinal Bacilli: EUGENE C. HOWE.

Six hundred and thirty strains of intestinal bacilli were collected from stools of twenty-one individuals—mostly healthy men. These were subjected to the following quantitative tests: (1) Acid and gas formation in dextrose, lactose, saccharose, raffinose, levulose, mannite and dulcite. (2) Acid production in milk. (3) Digestion of starch. (4) Production of indol from peptone. (5) Reduction of nitrates. (6) Gelatin liquefaction. (7) Morphology. (8) Motility.

The resulting data were analyzed statistically with the following conclusions to date relative to the non-gelatin liquefiers (540 strains):

1. Confirmation of the accepted view that motility has not systematic significance within the group "*B coli* and closely related organisms."

2. Lack of classificatory value of amount of gas produced and "gas ratio."

3. Acid formation, a sounder criterion than production of gas.

4. Mannite, dulcite and starch of little value in classification of this group, in connection with the other tests used. There is no correlation between the first three and the latter.

5. Indol, ammonia, and nitrite formation but slightly correlated with the general robustness of the organism and of little significance in classification within this group.

6. Dextrose, lactose, saccharose and raffinose constitute a natural *metabolic gradient.* Fermentation of any member of the series implies fermentation of members lower in the series.

7. On this basis there are two main groups of dextrose fermenting, gelatin-minus, non-spore-forming, intestinal bacilli and four sub-groups.

I. (58 %) { Dex. + Lact. + Sac. + Raf. + 1 (53 %)
{ Dex. + Lact. + Sac. + Raf. — 2 (5 %)

II. (42 %) { Dex. + Lact. + Sac. + Raf. — 3 (41 %)
{ Dex. + Lact. — Sac. — Raf. — 4 (1 %)

The Green Fluorescent Bacteria of Maple Sap: H. A. EDSON and C. W. CARPENTER.

Green fluorescent bacteria are the most important agents in the deterioration of maple sap. These microorganisms feed upon the traces of protein present in the sap, but have little, if any, action upon the sugar. The sap becomes cloudy with more or less green color and produces an inferior quality of syrup and sugar.

Forty-two strains of this group of bacteria which were isolated from maple sap, together with five cultures of known species from Kral and one from Novy, were studied. The latter were: *B. fluorescens albus, B. fluorescens liquefaciens, B. fluorescens longus, B. fluorescens mesentericus, B. fluorescens tenuis* and *B. fluorescens putidis.* The chief differences observed in the entire series of cultures were in respect to the following characters: nitrate reduction; growth on synthetic media; gelatin liquefaction and casein digestion in milk; hydrogen sulphide production; temperature relations.

Thirty-three strains of the fluorescent sap bacteria agree closely with *B. fluorescens liquefaciens;* two strains resemble *B. fluorescens mesentericus* and seven strains are similar to *B. fluorescens tenuis.*

Bacterial Variation Due to Acidity and Flow in the Youghiogheny River at McKeesport, Pennsylvania: E. C. TRAX.

The germicidal action of drainage from coal mines, containing as it does free sulphuric acid and iron in solution, is indicated by its composition.

Experiments made by the Department of Health of Pennsylvania lead to the conclusion that "Mine water will prevent the growth of typhoid bacilli after the lapse of one hour, and will markedly limit the growth of colon bacilli so that they die off progressively and can not be cultivated after 24 hours."

The acidity of the water in the Youghiogheny River is caused by the acid mine drainage, an immense quantity of which is discharged into the river and its tributaries. The reaction of the

water at McKeesport ranges from 20 parts per million alkaline during high stage of water to 39 parts acid at low stage, and the bacterial life of the stream is directly affected thereby.

The monthly averages of bacteria per cubic centimeter, acidity and height of river, are given below for the year 1910:

Month 1910	Av. Acidity (a)	No. Bacteria per c.c. (b)	Stage of River (c)
January	11	31,000	5.3
February	37	20,000	3.4
March	36	21,000	3.1
April	52	12,000	1.8
May	23	2,000	1.6
June	6	6,500	3.3
July	65	205	0.7
August	182	9	0.1
September ...	113	97	— 0.2
October	240	240	— 0.1
November	176	160	0.1
December	29	2,400	2.0

(a) Acidity to methyl orange in parts per million.

(b) 48 hours' incubation at 20° C.

(c) Gauge height in feet at West Newton.

It can be stated in a general way that the bacterial numbers vary with the gauge height of the river and inversely as the acidity. The acidity of the water is controlled by the conditions of rainfall, run off and flow, inasmuch as these are the factors which affect the dilution of the mine drainage. Allowing for the natural fluctuation of bacterial life in a flowing stream, the presence of the mine water is responsible for a considerable reduction at all times except during floods, when the water is alkaline, while during high acidity the effect approaches sterilization.

Water Sterilization by Emergency Chlorinated Lime Treatment Plants: RALPH E. IRWIN.

When emergencies call for the immediate sterilization of a public domestic water supply, temporary treatment apparatus may be constructed by using barrels to mix and feed chlorinated lime into the suction main, suction well or point where the water passes and thorough mixing is insured.

The solution may be mixed and settled in one barrel and fed from another *via* regulating valves. With this crude device water from large and small streams, wells and springs have been disinfected and communities protected from water-borne disease.

Two examples are given showing the bacteriological results obtained by treating similar spring waters that were infected and had caused epidemics of a water-borne disease.

The first spring furnished 1 to 1.5 million gallons daily and was under municipal control where political protection was given inefficient employees. During a period of 115 days, bacteriological determinations were made showing the total number of bacteria and *B. coli* present in 85 samples of untreated water from the spring, 70 samples of treated water as it left the pump and 75 samples from taps about the city. On 8 days samples were obtained showing *B. coli* in such large numbers that it was evident little, if any, lime was being added. The results as a whole show, however, that the prescribed 6 to 8 pounds of high-grade chlorinated lime per million gallons was sufficient to sterilize the water if added as directed.

The second spring furnished 3 to 3.5 million gallons daily and was under strict corporate control with employees obeying orders. During a period of 103 days, bacteriological determinations were made showing the total number of bacteria and *B. coli* present in 36 samples of untreated water from the spring and 36 samples of treated water from taps on the pump or distributing system. The treated water showed excellent reductions in total counts in every instance, and *B. coli* were absent throughout the period of treatment.

With a crude device such as described in the hands of efficient workmen during emergencies, creditable results may be obtained and valuable protection given.

The Distribution of Bacteria in Certain New York Soils: H. J. CONN.

Extensive work for two years with a certain clay loam at Ithaca has resulted in the isolation and study of about five hundred cultures. These cultures have been classified into thirty-four types, which are essentially species. Grouping these types into six easily distinguished classes, their relative frequency can be thus stated:

5–10 per cent. spore-producing liquefiers; large rods (*e. g., B. subtilis* and *B. mycoides*).

5–10 per cent. non-spore-producing, rapid liquefiers; small rods with polar flagella (*e. g., Ps. fluorescens*).

40–70 per cent. non-spore-producing, slow liquefiers; short rods, immotile (except one with polar flagella); growing very poorly in ordinary laboratory media.

Ca. 10 per cent. non-spore-producing, non-liquefiers; short rods, immotile or with polar flagella.

Trace. Micrococci, like the last group physiologically.

15–45 per cent. Actinomycetes.

Of these six groups all are strict aerobes except a few in group 1; almost without exception none produce gas from sugars; while acid production, although common, is always very weak.

Each group comprises about seven or eight types, except the last two, in which there are but one or two types.

This year forty more cultures have been isolated from four other soils elsewhere in the state. Two were clays, one a silt and the other a sand. With few exceptions these cultures seem to be the same kinds as those previously studied, although the relative frequency of the types is different. This suggests that there is a characteristic bacterial flora of soil. Accordingly, an intelligent comparison of soils demands the development of a technique to determine the relative abundance of the various kinds of organisms.

Soil Organisms which Destroy Cellulose: KARL F. KELLERMAN and I. G. MCBETH.

Our knowledge of cellulose destruction in soils is inadequate. Omeliansky's conclusions that cellulose is destroyed only under anaerobic conditions and gives rise either to hydrogen or methane are erroneous.

Two species of cellulose-destroying and five species of contaminating bacteria were isolated from Omeliansky's hydrogen culture, and one cellulose-destroying and two contaminating forms from his methane culture; none of the three species showed any resemblance to Omeliansky's hydrogen or methane ferments. In addition to the species isolated from Omeliansky's cultures eleven other species have been isolated from various other sources, one of which belongs to the thermophile group.

Contrary to Omeliansky's observation that cellulose-destroying bacteria do not grow upon solid media, most of the species isolated were found to grow readily upon such media as beef agar, gelatin, starch, potato and dextrose. Some of them have the power to liquefy gelatin. Although several of these organisms were isolated under anaerobic conditions, they grow equally well or better in the presence of air, which shows that the destruction of cellulose by bacteria is an aerobic rather than an anaerobic process.

It is usually supposed that filamentous fungi are of little importance in agricultural soils; these investigations show them to be at least as important as bacteria in destroying cellulose. About seventy-five species of molds have been isolated representing a large number of genera; species of *Penicillium, Aspergillus* and *Fusarium* are perhaps most numerous.

In the destruction of pure cellulose either by bacteria or molds in synthetic media the associative action of organisms which presumably have no cellulose-dissolving enzymes frequently stimulates the growth of the cellulose organism and increases its destructive power.

Nitrates in Soils: F. L. STEVENS.

Nearly all text-books assert that nitrates are the chief source of nitrogen supply for green plants. Recent experiments throw doubt on this assertion. Attention was called to the need of tests bacterially and chemically controlled, conducted under natural conditions, to determine what forms of nitrogen are most readily available to the leading crop plants. Nitrification and denitrification were discussed. In particular question was raised as to the influence of organic matter mixed with nitrates in fertilizers (a common practise) upon loss by denitrification. Stress was laid upon the need of conducting tests in soils, not in solutions.

Why do some Soils Nitrify Organic Nitrogenous Substances and the Ammonium Salts of Organic Acids Faster than they do Ammonium Sulphate or Ammonium Chloride? J. C. TEMPLE.

Of 26 Georgia soils tested for nitrification, 24 were found to nitrify tankage more readily than ammonium sulphate, in some cases the amount of nitrate recovered from tankage was ten times that recovered when ammonium sulphate was the source of nitrogen. Tankage, cotton-seed meal, cowpea vines, gelatin, peptone, asparagin, urea, ammonium citrate, ammonium oxalate, ammonium tartrate, ammonium bicarbonate and ammonium hydrate were nitrified faster than ammonium sulphate or chloride. This condition was not due to the nature of the nitrifying organism in the soil, as the same thing held true when the nitrifying organisms were supplied as pure cultures, obtained from a number of sources. When calcium carbonate was added to the soil, ammonium sulphate was nitrified as well as any of the other substances.

The explanation offered for this condition was that these soils (all of the Cecil group) were acid, and that the soil organisms decomposed the substances of organic origin in a way that more ammonia than acid was produced, thus correcting

the acidity and bringing about a condition favorable for the growth of the nitrifying organisms. When ammonium sulphate or ammonium chloride was added to the soil there was no chance for a similar decomposition and the soils remained acid.

Bacteriological Studies of the Fixation of Nitrogen in Certain Colorado Soils: WALTER G. SACKETT.

The power to fix atmospheric nitrogen is a property common to many cultivated Colorado soils.

This power is not confined to the fixation of nitrogen in solutions, but is manifested in soils as well.

"The rate of fixation of nitrogen obtained is sufficient to account for the nitrates found in the soil provided that it is nitrified. The rate of nitrification obtained is sufficient to account for the formation of the nitrates found in most cases, if not all of them."

The nitrates formed are sufficient to destroy all vegetation, in one case amounting to 172 tons per acre in the surface five inches.

The nitrogen-fixing power is not confined to any geographical locality or class of soils, however, the adobe shale soils, both in a raw state and when newly cultivated, possess little, if any, nitrogen-fixing power.

Excessive nitrates either destroy or greatly attenuate the nitrogen-fixing flora of a soil.

A limited amount of soil nitrate does not seriously affect the nitrogen-fixing power of a soil.

Azotobacter chroococcum appears to be the dominant nitrogen-fixing organism in the soils studied.

The dark brown color of the niter soils is due, in a large part, to the pigment produced by *Azotobacter chroococcum.*

Given a source of energy, the nitrate is the limiting factor in the production of the brown color.

In the presence of nitrates, *Azotobacter chroococcum* develops a chocolate brown to black pigment; nitrites, in certain amounts, produce similar results, but to a less degree; nitrogen as NH_4Cl, $(NH_4)_2SO_4$, asparagin, and peptone has no effect upon this function.

The highly colored extracts obtained from certain niter soils suggests that the pigment of *Azotobacter chroococcum* may be soluble in the alkaline soil waters.

Excessive soil moisture, by interfering with the growth of *Azotobacter chroococcum,* prevents the formation of the brown color on the soil, and makes the fixation of atmospheric nitrogen impossible.

Excessive irrigation, too diligent cultivation and the alkaline reaction of our soils appear to favor unduly the growth of *Azotobacter.*

This paper is published in full as Bulletin 179 of the Colorado Experiment Station, Fort Collins, Colorado.

The Movement of Nitric Nitrogen in Soil: ROBERT STEWART and J. E. GREAVES.

In the work which has been conducted for eight years at the Utah Experiment Station upon the influence of irrigation water upon the production and movement of nitric nitrogen in the soil, there has been observed a variation in the nitric nitrogen content of the soil and the concentration of the soil solution with the water applied, the crop grown and with the season.

The soil upon which these investigations have been conducted is ideally adapted both chemically and bacteriologically to support a rapid bacterial action, yet the amount of nitric nitrogen present to a depth of ten feet does not exceed three hundred pounds per acre.

Deposits of nitrates do occur in the country rock in widely distributed areas in western America.

The careful analytical work reported by Dr. Headden on the composition of Colorado soils indicates a close relationship between the nitric nitrogen and chlorine content of these soils, indicating clearly a common origin of these two elements.

The Present Status of Soil Inoculation: KARL F. KELLERMAN.

The method of pure-culture inoculation is less certain than the use of soil from old well-inoculated fields, but has, however, the advantage of cheapness and greater ease of transportation and application, as well as the important advantage of the absence of introducing weeds and plant diseases. The crown-gall disease of fruit trees is the most conspicuous example of disease which may be disseminated by soil transfer.

Reports received from farmers who have conducted inoculation tests with cultures distributed by the Department of Agriculture during the past seven years give an average of 76 per cent. success and 24 per cent. failure, if only those reports are considered that make possible some determination regarding the action of cultures. If previously inoculated fields, crop failures and such other doubtful cases are included with the failures our percentage of success for this same period is reduced to 38.

The organism producing nitrogen-fixing nodules on the roots of legumes has been isolated and cultivated since 1903; di Rossi's contention that the proper organism had not been isolated prior to his work in 1907 appears without foundation.

By a new technique it has been possible to stain the flagella of this organism. Instead of bearing a single polar flagellum it is supplied with several peritrichic flagella. The proper designation of this organism, therefore, is *Bacillus radicicola.*

The Persistence and Vitality of Bacteria on Alfalfa Seed: M. J. PRUCHA.

The seeds of the common farm crops such as wheat, corn, peas, alfalfa, etc., are extremely difficult to sterilize without killing the seed. It has also been shown that the bacteria of disease are carried on beans and corn. It is important to know to what extent bacteria may persist on the seed.

The following results were obtained from a quantitative and qualitative study of alfalfa seeds.

Nineteen samples, grown and collected in 1909, from 11 different states, have been studied for two years.

On fresh seed the germ content varied from 16,000 to 12 per seed. With age the germ content decreases. A typical sample which when fresh had 7,780 per seed, when two years old gave 340 bacteria per seed.

Simultaneous platings were made from the 19 samples and representatives of each apparent group were determined according to the Society Card.

Of the 84 different group numbers determined, 35 were Bacillus, 21 Bacterium, 19 Pseudomonas, 1 Streptococcus and 8 Yeast. About one third of these forms were widely distributed and many of them very persistent on the seeds. Of the 84 groups, 68 were chromogenic, yellow being much the more common. The samples from semi-arid regions gave especially brilliant colors. But 8 of the 84 groups were spore formers and the spore formers represent only about one fifth of the forms present at the end of two years.

The reduction in numbers of bacteria, with age, is due to a decrease within each group, gradually the less numerous groups disappear. At the end of two years the most widely-distributed and most numerous group is Bact. 211.3332533—a non-spore former.

This work will appear as a bulletin of the New York Agricultural Experiment Station, Geneva, N. Y.

The Behavior of Pseudomonas radicicola in the Soil: B. M. DUGGAR and M. J. PRUCHA.

This paper is in the form of a preliminary report on (1) the effects of conditions, especially drying, on the vitality of the germ, and (2) the multiplication of the germ in soil under the influence of various factors. The results indicate that there are certain undetermined factors which seem to affect vitality after drying, yet it seems certain that after the rapid or sudden drying-out of soil cultures there remains a considerable number of living organisms, the existence of which may be determined either by the direct plate method, indirect plating (after inoculation into bouillon) or host inoculation. When soil cultures are directly and rapidly dried out the number of organisms found by the plate method may be no more than about one twentieth of those present when the drying began. This, however, relates to excessive drying. Where the drying process is less complete, the number remaining alive is much greater, and the life of the germ extends over a considerable period of time.

Cultures of this germ in sterile soil (clay loam) after five days gave about 160,000,000 organisms per gram, which is considerably more than the number found per c.c. in a control bouillon culture. In certain experiments, sterile and unsterile soils were mixed in various proportions, and the mixed material thoroughly inoculated and compared with the check in sterile soil. The addition of the unsterile soil inhibits multiplication of the legume germ as the amount of unsterile soil is increased.

Casein Media Adapted to Milk Analysis: S. HENRY AYERS.

CASEIN AGAR

Preparation of One Liter

Casein Solution	Agar Solution
300 c.c. distilled water	500 c.c. distilled water
10 gm. casein (Eimer and Amend c.p. casein prepared according to Hammersten)	10 gm. agar
7 c.c. normal sodium hydroxide.	
After dissolving casein make up to 500 cubic centimeters.	

To 300 c.c. water (distilled) add
10 gm. casein (Eimer and Amend c.p. casein prepared according to Hammersten)
and 7 c.c. normal sodium hydroxide.

Dissolve casein by heating to boiling. It is desirable to let this solution stand for several hours to get a perfect solution. This is not necessary, however. Make up volume to 500 c.c. and bring the reaction of the solution to between + 0.1 and + 0.2 Fuller's scale. Do not allow this solution to become alkaline to phenolphthalein or over — 0.2. If the casein is weighed accurately and the normal solution accurate the reaction will be about + 0.2.

The agar solution is prepared by dissolving 10 gm. agar in 500 c.c. of water. Both casein and agar solution should be filtered, then mixed. Tube and sterilize in autoclave under pressure for 20 minutes; then cool the tubes quickly in cold water or ice water. The final reaction of the medium will be about + 0.1 Fuller's scale. If the medium is alkaline, the bacterial growth will be restricted. If the medium is more than + 0.1 some of the casein may be precipitated during sterilization. The casein agar should be clear and almost colorless when poured in a Petri dish. Sometimes the casein will be slightly precipitated during sterilization on the cooling, but it is of no consequence, since on pouring into plates the precipitate on account of its finely divided condition becomes invisible.

The study of the bacterial growth on casein agar and infusion agar shows the following points:

1. The 24 hours' count at 37° on casein agar was almost always lower than on infusion agar when raw milk is being examined. When pasteurized milk was examined the casein plates showed a higher count in 37 per cent. of the samples.

2. After 6 days' incubation at 30° C., out of 50 samples of raw milk plated, 44 per cent. of the samples showed higher counts on casein agar. With 50 samples of pasteurized milk, 78 per cent. of the samples showed a higher count on casein agar.

3. From a study of the bacteria from about 50 samples of both raw and pasteurized milk it seems that acid-forming bacteria do not develop quite as well on casein agar. It does, however, favor the growth of the alkali formers, the peptonizers and inert bacteria.

4. The number of peptonizing bacteria in a sample of milk may be determined directly from a casein agar plate. After counting the plate it should be flowed with $N/10$ lactic acid; this causes the precipitation of the casein, giving a white opaque plate except where the casein has been dissolved about a colony of peptonizing bacteria. There is then left a clear zone around the colonies of peptonizing bacteria which enables one to determine their numbers in the sample of milk under examination. It has been found from a study of a large number of samples that this method of determination is accurate.

Sugars may be added to the casein agar or the casein solution may be used as a liquid medium without agar. It is believed that these media using casein will be of considerable value in bacteriological milk analysis.

The Analysis of the Gases Produced by One Hundred Cultures of Bacteria: WM. MANSFIELD CLARK.

The purpose of these analyses was to furnish data for the identification of gas-producing bacteria isolated from dairy products.

The bacteria were grown in a special form of culture bulb, evacuated with a mercury pump after inoculation, sealed up and incubated seven days at 30° C. The culture medium was a bouillon containing 1 per cent. dextrose. Exactly 5 c.c. of this was used in each bulb.

The collection of the gas was made with an Antropoff mercury pump and the analyses were made with special burettes and Hempel pipettes adapted for accurate analyses of small volumes.

The majority of the cultures analyzed gave a ratio of CO_2, H_2 similar to that of *B. coli communis*. Certain other distinct ratios were found. These depend in large measure upon the volume of CO_2, the hydrogen tending to remain constant. Certain other relationships are suggested tentatively, pending further investigation.

A Study of Gas-forming Bacteria in Milk: L. A. ROGERS and B. J. DAVIS.

Cultures of gas-forming organisms have been isolated from milk and other dairy products obtained in various parts of the country. These have been studied with special reference to the relation between certain physiological reactions, as the fermentation of carbohydrates and the amount of gas and ratio of H_2 to CO_2. Plotted on the frequency basis the $H_2 : CO_2$ ratio has given four more or less distinct nodes, one at the ratio 1 : 1.1, one at 1 : 1.8, one at 1 : 2.2, and one at 1 : 2.7.

Arranged in a similar way, the amount of gas produced under given conditions shows nodes at 4 c.c., between 7 and 8 c.c. and 17 c.c.

Proper classification of the cultures shows a close correlation between the $H_2 : CO_2$ ratio and the amount of gas.

The gas ratio is further correlated in some cases with the fermentation of certain carbohydrates.

The group giving a ratio of 1 : 1.6 to 1 : 2.0 shows a distinctly greater ability to ferment saccharose, raffinose and starch than the group giving the ratio 1 : 1.1. It is probable that these tentative groups are somewhat heterogeneous and that further refinement by the use of new test substances will bring out sharper distinctions.

The Bacteriology of Cheddar Cheese: E. G. HASTINGS and ALICE C. EVANS.

Will appear soon in bulletin form jointly from the Dairy Division, Bureau of Animal Industry, U. S. Department of Agriculture, and the Wisconsin Experiment Station.

Some Actions of Microorganisms upon the Constituents of Butter: CHARLES W. BROWN.

For this work one lot of cream, divided into two parts—one part pasteurized at 160° to 170° F., the other not pasteurized—was churned and the butter placed in storage at —3° F. to + 3° F. Of the 88 different species of microorganisms, not including molds or the higher bacteria, isolated from this butter 57 are bacteria (cocci, bacilli or spirilla) and 31 are yeasts. It was noticed:

1. That 24 of the bacteria and 15 of the yeasts will grow on 12 per cent. salt at 20° C. Four of these bacteria and six of these yeasts grow well on 12 per cent. salt at 6° C.

2. That the ratio of the number of species of liquefying bacteria to the number of non-liquefying bacteria isolated from ordinary agar is the same as the liquefying to the non-liquefying isolated from 12 per cent. salt agar.

3. That 12 per cent. of salt has a much more inhibitive action upon the species of liquefying yeasts than it does upon the non-liquefying.

4. That the lactose in both the pasteurized and unpasteurized butter decreased from 0.315 per cent. and 0.325 per cent. to 0.285 per cent. to 0.290 per cent., respectively, in 428 days.

5. That 50 per cent. of the decrease in lactose took place within the first 10 days.

6. That when the butter was taken from storage at the end of 428 days and placed at room temperature very little further decomposition of lactose occurred.

7. That the soluble nitrogen recorded in percentage of the total nitrogen in the butter increased in 428 days from 6.25 per cent. and 7.69 per cent. to 6.29 per cent. and 7.84 per cent. for the pasteurized and unpasteurized, respectively.

8. That the acidity of the pasteurized butter remained constant while that of the unpasteurized increased from 25.5° to 33.9° (Fuller's scale).

9. That when the growth upon synthetic agar was compared with the growth upon the same agar to which 1 per cent. butter fat—freed from impurities by melting and decanting—was added, 9 species of the bacteria showed a more luxuriant growth in the presence of fat, 11 were inhibited and 37 were indifferent; while 20 of the yeasts grew more luxuriant, 5 were inhibited and 6 indifferent.

A Bacteriological Study of the Milk Supply of Washington, D. C.: J. J. KINYOUN and L. V. DEITER.

A series of bacteriological examinations of the milk supply of Washington, D. C., were continued over a period of 14 months beginning in September, 1910, and ending on November 1, 1911. The objects of these examinations were to ascertain as near as was possible the actual conditions of the milk supply during this period so as to be able to formulate some means of its improvement.

Samples of milk were examined in accordance with the rules and methods prescribed by the Laboratory Section of the American Public Health Association and in addition thereto special methods were employed for the detection of the colon group.

The result of this study was that the milk supply of Washington was on the whole very unsatisfactory and was capable of a great improvement.

Nearly all the raw milk arriving in the city by rail had a very high bacterial content, the average for all samples for the 14 months was 9,300,000 and in no instance was it below 1,000,000.

55 per cent. of the samples contained both colon and streptococci. The close parallel between these two groups is looked upon by the writers as a sure indication of dirty collection and imperfect handling.

The examinations of the "pasteurized" milk as it is purveyed is far from satisfactory. This condition was due in a great measure to the imperfect way in which the process was applied, or to the attempts of the dealer to pasteurize an old or a dirty milk in order to sell it.

It has been clearly demonstrated by this study that a great amount of the milk as supplied is collected under unfavorable conditions, and is imperfectly or carelessly handled.

The Bacteriological Improvement of a Milk Supply by other than Laboratory Means: H. A. HARDING.

Bacterial studies have shown that the essentials for the production of cleaner milk are:

1. The utensils and the cow and her surroundings during the milking process must be as clean as possible.

2. The milk must be cooled as promptly and as thoroughly as possible. The problem of the bacteriologists becomes: how to induce the production of milk in accord with these essentials.

Attempts at securing this by establishing maximum permissible germ contents are undesirable because:

1. We lack data for establishing the point at which germ content begins to menace the public health.

2. We lack technique for determining the germ content of milk with an accuracy demanded by such legal enactment.

3. Such enactment has slight educational value because it can not be readily translated by dairymen into terms of their dairy practises.

The bacteriologists must translate the results of their studies into terms of dairy practises, and this translation may well take the form of a *score card.* If the valuation in this score card is correct the resulting score is an accurate measure of the relative desirability of the dairy product.

Such a mathematical expression is valuable because it facilitates buying and selling milk on the basis of quality.

In Geneva, N. Y., where the Cornell score card was taken voluntarily by the milkmen as a basis of payment according to quality:

"Poor" milk, originally one third of the total supply, decreased sharply and disappeared after three years.

"Medium" milk, originally about two thirds of the supply, decreased sharply and disappeared after three years.

"Good" milk, originally only five per cent. of the supply, quickly displaced the two lower grades.

"Excellent" milk, previously unknown, was twelve per cent. of the supply after three years.

The details of this work are given in New York Agricultural Experiment Station Bulletin 337.

This complete transformation of a municipal milk supply was accomplished at a cost to the city of $500 per year.

The dairymen are desirous of furnishing the highest grade of milk for which they can get a price proportionate to the quality. The first necessity is a definition of the desired quality in terms which the dairymen can clearly understand. The dairy score card is the most promising attempt in this direction. The second necessity is the establishing of definite market grades of quality in milk, so that the consumer can purchase intelligently and create a commercial demand for a better article. The action of the New York Health Department in this direction is commendable.

Any permanent improvement in a municipal milk supply must rest upon conditions which make it more profitable to furnish a cleaner milk than to furnish a dirtier one.

The Principle of Vacuum Cleaning as Applied to Dairy Cows: G. L. RUEHLE.

The Object.—A comparison of the results obtained by a vacuum cleaner and by hand cleaning of cows. The points considered were (1) the effect on the germ content of the milk, (2) the time consumed.

The Method.—Two cows were cleaned each night by each method. The groups were alternated on succeeding nights. Observations were made on 22 nights.

The general average for hand cleaning was 669 per c.c., and for machine cleaning it was 1,145 per c.c.

Time Consumed.—Owing to the small number of cows per day, measurements of the time required by each method were not satisfactory. However, it was plain that the vacuum cleaning consumed more time than hand cleaning. As vacuum cleaning of cows took more time and gave poorer results, it does not commend itself to dairy practise.

EFFECT ON GERM CONTENT

	Germ Content per c.c. from Machine and Hand Cleaned Cows							
	Cow No. 1		Cow No. 2		Cow No. 3		Cow No. 4	
	Hand	Machine	Hand	Machine	Hand	Machine	Hand	Machine
No. samples	10	12	10	12	11	11	11	11
Totals	20,765	26,459	1,479	1,624	2,541	16,309	3,305	8,297
Averages	2,077	2,205	148	35	231	1,483	300	754

Results will appear in a Bulletin of the New York Agricultural Experiment Station.

Suggestion of a New Method of Stating Composite Results of Bacterial Milk Counts: ERNEST C. LEVY.

Statement of the "average bacterial count" of milk samples in any city is of comparatively little value on account of the influence of a few samples, or even a single sample, of very high bacterial content.

The most approved method of statement of results has therefore been to give the number of samples, and the percentage of samples, falling in each of certain more or less arbitrary groups or classes, in the following manner:

(*A*) Class	No. of Samples	Per Cent. of All Classes
Under 10,000	25	16.7
10,001 to 50,000	73	48.6
50,001 to 100,000	37	24.7
100,001 to 250,000	9	6.0

If we apply this method to the hypothetical 150 samples given under (*A*) we get the following:

(*C*) Class	Rating Figure	No. of Samples in Each Class	Product
Under 10,000	100	25	2,500
10,001 to 50,000	90	73	6,570
50,001 to 100,000	75	37	2,775
100,001 to 250,000	50	9	450
250,001 to 499,000	20	3	60
Over 500,000	0	3	0
Totals		150	12,355
"Bacterial index"			82.4
250,001 to 500,000	3		2.0
Over 500,001	3		2.0
Total	150		100.0

This method, while of more real value than a mere statement of average count, is too cumbersome. In order to get around these difficulties, a new method of statement—the "bacterial index"—is suggested. To each of the groups above shown a rating value is given, as follows:

(*B*) Class	Suggested Rating Figure for Raw Milk
Under 10,000	100
10,001 to 50,000	90
50,001 to 100,000	75
100,001 to 250,000	50
250,001 to 499,000	20
Over 500,000	0

The bacterial index thus arrived at takes into account the number of samples falling in each class, but at the same time enables us to state our results in a single figure, and this figure is not unduly influenced by exceptional samples. The method itself is believed to be of real value, but the rating figures given are only suggestive and, if the method is adopted for general use, proper rating figures should be agreed upon after careful consideration by some competent body of bacteriologists.

In applying the method of statement to samples of pasteurized milk, a different set of rating figures should be used. We know less about this than about raw milk, but the following ratings are given as illustrative:

(*D*) Class	Suggested Rating Figure for Pasteurized Milk
Under 100	100
101 to 500	90
501 to 1,000	75
1,001 to 5,000	50
5,001 to 9,900	20
Over 10,000	0

An additional advantage of using the bacterial index in stating results for pasteurized milk samples is that we get around the danger of having misleading comparisons made between the bacterial counts of raw and pasteurized milk. Instead of this, with proper rating figures for each kind of milk, we can compare any group of raw samples with ideal raw milk and any group of pasteurized samples with ideal pasteurized milk.

The Control of Pasteurized Milk by Physical and Bacterial Standards: WILLIAM ROYAL STOKES and FRANK W. HACHTEL.

The article after emphasizing the importance of the control of the pasteurization of milk and of milk after it has been pasteurized described the bacterial reduction obtained through the pasteurization of milk by means of the so-called "slow" and "rapid" methods. It then mentioned the physical and bacteriological standards for the control of pasteurization which were established by Koehler and Tonney, of Chicago. The minimum temperature requirements for the continuous or rapid type of pasteurization are 160° F. (71° C.) for one minute, and for the slow or "holding" method 140° F. (60° C.) for twenty minutes. These requirements have been adopted

since the tubercle bacillus is destroyed under such conditions, and this is considered as a sanitary index of efficient pasteurization. As the bacteriological standard they require that there should be a reduction of 99 per cent. of the bacteria after pasteurization as compared to the raw milk, but this is not strictly applied if the bacteria are less than 100,000 per c.c. Koehler and Tonney have also shown the percentage of reduction during the various stages of pasteurization by the rapid method varying between 150° F. and 164° F., and by the slow method varying between 143° F. and 150° F. The bacterial count even in the bottled milk at the end of both processes showed a bacterial reduction of about 99.5 per cent., with the exception of the bottled milk in the rapid method, which only showed a reduction of 98.75 per cent.

This article, then citing the work of the authors, shows an average reduction by pasteurization in Baltimore of 99.4 per cent. by the rapid method and 99.1 per cent. by the slow method. There were fewer counts made of the rapid method (96) than by the slow method (146), and the counts of the raw milk by the rapid method were much higher.

The writers have also studied the percentage of cases in which the colon bacillus was present before and after pasteurization in 1 c.c. or in 1/10 c.c., and their results were as follows:

PERCENTAGE OF CASES IN WHICH COLON BACILLI WERE PRESENT BEFORE AND AFTER PASTEURIZATION

Rapid Method					Slow Method				
Number of Examinations	Colon Bacillus Present Before Pasteurization in 0.001 c.c.		Colon Bacillus Present After Pasteurization in 1 c.c.		Number of Examinations	Colon Bacillus Present Before Pasteurization in 0.001 c.c.		Colon Bacillus Present After Pasteurization in 1 c.c.	
96	45	46.8 %	48	50.0 %	146	86	58.9 %	87	59.5 %
Number of Examinations	Colon Bacillus Present Before Pasteurization in 0.001 c.c.		Colon Bacillus Present After Pasteurization in 0.1 c.c.		Number of Examinations	Colon Bacillus Present Before Pasteurization in 0.001 c.c.		Colon Bacillus Present After Pasteurization in 1 c.c.	
33	22	66.6 %	7	21.2 %	93	68	73.1 %	42	45.1%

The article then considers the recontamination of pasteurized milk, showing by the work of Koehler and Tonney that while the average count from a large number of freshly pasteurized milks was only 125,000, yet the average count from pasteurized milk one day old was 602,000 bacteria per cubic centimeter. Some of this milk showed counts varying between 1,000,000 and 4,800,000 per cubic centimeter. These authors think that this recontamination can best be obviated by a strict enforcement of a maximum standard for the temperature of milk of 50° C.

The conclusions are that the physical and bacterial standards of Koehler and Tonney are reasonable, and that the question of an additional safeguard establishing a maximum amount in which colon bacilli can be present in pasteurized milk is still open for debate.

Recent Developments in Pasteurization of Milk for a General Market: EDWIN HENRY SCHORER.

Pasteurization is employed legitimately to destroy pathogenic organisms of diseases transmitted through milk and to preserve milk so that it may be transported when properly refrigerated to localities where fresh milk is not obtainable. The process is used fraudulently to give low bacterial count to dirty milk, a redemption process, and to make milk keep in a manner similar to that of carefully obtained milk. In any event the process depends on heating milk to a temperature for a sufficient period of time to destroy the offending microorganisms. For fraudulent purposes it is only essential that a large percentage of bacteria be destroyed, while if milk is to be rendered free from possibility of causing infection, it is imperative that all pathogenic organisms be killed.

The entire process is based on scientific investigation, but unfortunately the results obtained in the laboratory are not obtained in the pasteurization of milk for the market. Pasteurization of market milk must either be done in the bulk before bottling and capping or else in sealed bottles. Bulk pasteurization does not prevent reinfection and pasteurization in the bottle is expensive and time consuming.

While the primary object of the pasteurization of milk should be to destroy pathogenic bacteria, determination of the accomplishment of this object is a relatively difficult and slow process. For this reason the reduction in numbers of bacteria in milk is taken as evidence of efficiency of pasteurization. It is generally claimed that pasteuriza-

tion kills the lactic acid organisms and leaves the spores of peptonizing and putrefying bacteria. In the United States, however, pasteurized market milk coagulated sooner than does certified milk and peptonization occurs more frequently in the best grades of raw milk than in pasteurized market milk.

It can not be hoped that pasteurized dirty milk can be made as good as pasteurized clean milk, nor can a uniform product be expected as the result of pasteurization of market milk. While the higher grades of raw milk quite consistently have a low bacterial count, still they show a marked variation in flora. This same variation is observed in pasteurized market milk.

The technique in the laboratory does not prevail in the dairy and while pasteurization in sealed bottles can be made to represent laboratory methods, pressure for time may lead to over- or underheating and shortening of the length of time of pasteurization. While heating to 140° F. for twenty minutes is sufficient in the laboratory to destroy pathogenic organisms, commercial conditions and mechanical devices are such that pasteurization should be carried on at a higher temperature and for a longer period of time.

The most efficient method of pasteurization is that under official supervision, controlling the quality of the milk pasteurized, pasteurization in the sealed bottle at 145° F. for thirty minutes, allowing at least thirty minutes to heat the milk to the pasteurizing temperature, and labeling such milk properly. This will insure sufficient temperature to destroy pathogenic bacteria, will inactivate the ferments but little, leave a good cream line and give a preferred milk.

The Bacterial Content of Oysters in the Shell in Storage: G. W. STILES.

Samples of oysters in the shell were taken from known localities during the months of November, 1910, and February, 1911, and placed in storage at a temperature of 39° F. Bacteriological analyses were made on five oysters constituting a single sample at various intervals ranging from one to 29 days. The usual technique for oyster work was observed for making the cultures. The oyster shells were well scrubbed in running tap water, their beaks briefly immersed in boiling water, and, with sterilized forceps and knife, the liquor removed from the shell into five sterilized petri dishes, one for the liquor of each oyster.

Definite quantities of 1 c.c., 0.1 c.c. and 0.01 c.c. from each oyster were planted into Durham fermentation tubes containing lactose peptone ox bile. Dilutions of one to one hundred, one to one thousand, one to ten thousand, etc., were planted in duplicate on agar plates and incubated at 20° C., and 37° C., respectively, for three days.

The results obtained from the fermentation tests were expressed according to the suggested score of the Committee on Standard Methods of Shellfish Examination (*Journal of American Public Health Association*, Vol. I., No. 8, August, 1911).

Charts illustrating the *B. coli* content and the total bacterial count were exhibited. The results obtained varied, but not sufficiently to give positive indications as to results which may be obtained with samples stored at higher degrees of temperature. The variation of individual samples not in storage may, under certain circumstances, be as great as the results obtained on those kept in storage. Additional work on oysters showing greater degrees of pollutions and during different seasons of the year are contemplated before final publication.

Seasonal Variation in the Bacterial Content of Oysters: GEO. H. SMITH.

During the sanitary survey of Narragansett Bay, carried on during 1910–11, several facts were brought out regarding the bacterial content of oysters during different seasons. Frequent examinations of several layings were made during the period from December, 1910, to May, 1911, that is, during the coldest part of the year and during the period of the warming of the water. In these examinations the total count of organisms in the shell-juice of the oyster and the sea-water, the presence or absence of the colon bacillus in the oysters and the sea-water, the temperature of the water, etc., were noted.

An analysis of the data thus obtained seems to warrant the following statements:

During the colder winter months both the total number of organisms and the number of *B. coli* present in the oyster are very low as compared with that of the warmer weather.

The times when the total count and the colon count of the oysters are lowest are not the same, except within wide limits.

The period when the oysters contain the lowest total number of organisms or the fewest *B. coli* is not the period of lowest temperature.

The drop in total bacteria and in colon is apparently not related in any way to the decrease of organisms in the sea-water.

Thus it appears that the decrease in the bac-

terial content of the oyster is not directly dependent upon the number of organisms present in the sea-water, nor is it the direct effect of temperature, but is rather due to some cause lying within the oyster itself. Possibly during the colder months the oyster enters a state of hibernation, when no bacteria are taken in and those already within are gradually eliminated.

Variation in Acid Production of Colon Bacilli from Different Sources: WILLIAM W. BROWN.

During the sanitary survey of Narragansett Bay, Rhode Island, under the direction of Professor Gorham, oysters were taken from 242 stations to determine whether the distance of the oysters from the source of the pollution had any relation to the amount of acid the colon bacillus was able to produce in dextrose and lactose broths, with the hope of determining whether pollution was recent or remote. But the factors governing such an experiment as the tides, currents and winds were so variable that the author could only draw a comparison between the amount of acid produced by colon freshly isolated from feces and the colon isolated from oysters. Oysters were taken from 242 stations in the bay, some located in badly polluted areas, while some were taken from conditional zones (sometimes colon-positive, again colon-negative). In all cases the oysters were examined according to the standard methods of the committee of the Laboratory Section of the American Public Health Association. The fecal colon were isolated from the stools of Italian immigrants. The dextrose and lactose broths used in the experiment were made according to standard methods, except that meat extract was used in all cases.

Titrations were made with $N/20$ sodium hydroxide into boiling solutions.

From the titrations it was noted that:

1. The optimum temperature for acid production by the colon bacillus is 37° C.

2. No matter how great the quantity of medium inoculated with the colon bacillus, the same per cent. of acidity is obtained.

3. More acid is produced in dextrose broth than in lactose broth by the colon bacillus.

4. The amount of sugar present in the medium has a direct relation to the amount of acid produced.

5. *Bacillus coli* freshly isolated from feces produces more acid in dextrose and lactose broth than *Bacillus coli* isolated from oysters.

Mutations in Microorganisms: D. H. BERGEY.

The success which seems to have attended the attempts to produce mutation in some of the animal organisms, especially Trypanosomes, stimulated the hope that it would be possible to produce similar mutations in bacteria. In fact such mutations have been encountered, notably those described by Neisser, Massini and others. I have selected a typical colon bacillus, isolated from feces, as a desirable organism with which to attempt to produce mutation forms. The success which Altmann and his associates had in changing the immunity reactions of the colon bacillus, suggested that it might be possible to change some of its other characteristics. With this object in view, I exposed the colon bacillus to various organic and inorganic substances, added to ordinary bouillon for varying periods of time. I then tested these organisms as to their general biological characters and the immunity reactions. Among the substances to which the culture was exposed were copper sulphate, chloroform, picric acid, resorcinol and horse serum. Thus far none of the biological characters of the organism have been altered—either increased or decreased to any appreciable degree. The immunity reaction, especially the agglutination reaction, appears to show evidences of alteration. These alterations are most marked in the race exposed to horse serum, though all of the chemicals employed seem to so change the organism as to inhibit agglutination altogether or permit its manifestation only in serum of high agglutinative power. The alterations in the characters of the organism are insufficient in degree to warrant one in classifying them as mutations.

The Antiseptic and Bactericidal Properties of Egg-white: LEO F. RETTGER and JOEL A. SPERRY.

Normal egg-white possesses marked antiseptic and bactericidal properties towards certain bacteria, particularly the members of the *subtilis* group. *B. subtilis* and *B. megatherium* were destroyed almost instantaneously when introduced into test tubes containing the whites of hens' eggs. Certain strains of *B. coli* and *B. typhi* were quite susceptible, while others were but slightly affected. The same thing was found to be true of *B. pullorum* (Rettger). With respect to the last-mentioned organism the degree of resistance bore a definite relationship to the virulence, the more virulent strains showing the greater resistance to the action of the egg-white.

Proteus Zenkeri was quickly destroyed, while *Proteus vulgaris* was less rapidly influenced and *Proteus mirabilis* suffered but little, if any.

Staphylococcus pyogenes aureus and *B. fluorescens* were very quickly affected. *Bacillus putrificus* (Bienstock) and *B. edematis maligni* were unable to develop and bring about any putrefactive changes whatever in the white of egg, while the yolk and the coagulated egg-meat medium rapidly underwent putrefaction.

Heating at 65–70° C. for 15 minutes destroys the antiseptic and bactericidal properties of egg-white.

The Effect of Certain Antiseptics upon Staphylococcus pyogenes var. aureus: T. D. BECKWITH, T. D. BRANDENBERG, J. DINWIDDIE and C. H. HOFSTRAND.

In order to learn the efficiency of certain antiseptics commonly used in veterinary practise the following work was carried out under test-tube conditions.

The antiseptic was added to distilled water with the proper dilution and a loopful of a twenty-four hour culture in broth of *Staphylococcus pyogenes* var. *aureus* isolated about one week previously from a case of empyema was then mixed with the antiseptic solution. Plates were poured, using standard beef agar after intervals of one, five and twenty minutes, all results being obtained in duplicate. The following seventeen compounds and preparations were used: alcohol, pyoktanin blue, pyoktanin yellow, Lugol's solution, potassium permanganate, cresol, liq. cresolis comp., boric acid, lysol, formaldehyde, hydrogen peroxide, silver nitrate, mercuric chloride, phenol, "Benetol," "Creolin," "Septico," the last three being preparations on the market.

Following appeared to be the order of efficiency, beginning with the most active: silver nitrate, mercuric chloride, Lugol's solution, pyoktanin blue, potassium permanganate, pyoktanin yellow, Liq. cresolis comp., cresol, "Benetol," "Creolin," formaldehyde, lysol, "Septico," hydrogen peroxide, phenol, boric acid, alcohol.

Check tubes and counts were made at frequent intervals.

Aggressin Immunization Against Symptomatic Anthrax: OTTO W. SCHÖBL.

Briefly, the results of the experiments are as follows:

1. The existence of aggressin in black leg edema has been proved, since the sterile edema fluid aids infection by hindering the natural protective apparatus of the organism. Phagocytosis chiefly is inhibited.

2. It is non-toxic even in much larger quantities than the amount necessary to change a sublethal dose of symptomatic anthrax bacilli into the lethal dose.

3. Repeated injections of sterile edema fluid leads to a considerable degree of immunity. The animals are not only immune themselves, but also yield serum that protects normal animals from subsequent infection.

4. Such a serum shows the presence of antibodies demonstrable both in vitro and in vivo, the most striking characteristic being its favorable effect upon the phenomenon of phagocytosis.

5. In the subcutaneous circumscribed infiltration following artificial infection, immunized animals may under certain circumstances harbor virulent symptomatic anthrax bacilli.

Therefore, the immunity can not be considered bacteriolytic.

6. The immunity consists of a complete or partial inhibition of the growth of symptomatic anthrax bacilli in the body of the immunized animal. If the immunity is not sufficient to suppress completely the growth of bacilli, they multiply locally and are still able to produce toxin. The difference between antitoxic and anti-infectious immunity is in the case of symptomatic anthrax quite evident.

7. The fact frequently observed in our experiments that immune animals may harbor in their bodies symptomatic anthrax bacilli, fully virulent for normal animals, is worthy of consideration from an epidemiological standpoint.

8. The method of immunization with aggressin is advantageous in that the inoculating material is a sterile fluid, hence the danger of making bacillus-carriers or setting up a virulent infection through the vaccinating material is avoided.

The Rôle of Homologous Cultures in the Production of Immunity in Rabbits to Fowl Cholera: PHILIP B. HADLEY.

The present paper describes certain aspects of an immunity artificially produced in rabbits against infection with a very virulent culture of the fowl cholera organism. This was accomplished by means of a subcutaneous inoculation with a small amount of a nearly virulent, homologous culture of the cholera bacterium. The following points are made clear:

1. Of ten different strains of the fowl cholera organism employed, only one (Culture 52) was capable of producing immunity to a highly virulent culture.

2. The smallest amount of Culture 52 found to produce immunity was 0.000,000,01 c.c., but

amounts as large as 3 c.c. were easily tolerated and gave similar results.

3. In protectively inoculated rabbits a slight resistance was manifested within 3 to 4 days, but complete immunity did not appear until the seventh day after the protective inoculation.

4. That the resistance in question was not a "zonal" or local immunity was shown by inoculation of previously protected rabbits in the ear, flank and back; also by intravenous and intraperitoneal inoculations, none of which were fatal.

5. The resistance produced by inoculation with 2 c.c. of Culture 52 was sufficient to protect against at least 3 c.c. of the virulent culture when the M.L.D. of the latter was one hundred-quintillionth (0.000,000,000,000,000,000,01 c.c.) of one cubic centimeter.

6. Both fowls and pigeons have been rendered immune by inoculation with Culture 52. But, whereas a single inoculation is invariably sufficient for rabbits, fowls and pigeons may require two.

7. Attempts to produce resistance by inoculation with killed and attenuated cultures have failed; likewise attempts to produce resistance by successive inoculation with gradually increasing numbers of living organisms; this last is probably due to the fact that inoculation with even 4 organisms is fatal.

8. In rabbits, immunity to the virulent culture has been found to endure for at least 8 months.

9. The immunity obtained through inoculation with Culture 52 is inherited; female rabbits, six and one half months after the protective inoculation, are able to give birth to young which are perfectly immune.

10. The blood serum from resistant rabbits is protective.

Serum Diagnosis of Glanders: JOHN R. MOHLER.

In 1909 Schütz and Schubert published the results of their important work on the application of the method of complement fixation for the diagnosis of glanders. Their experiments were followed by splendid results, exceeding by far those obtained by either the mallein or agglutination test. Consequently, they recommended that this method of diagnosis in combination with the agglutination test be taken as the official test in Germany. This method, overcoming as it does the disadvantages of the mallein and agglutination tests, constitutes the most reliable method for the diagnosis of glanders which we have at our command at the present time. It has recently been thoroughly studied by the Bureau of Animal Industry and has proved to be highly satisfactory. The principle of this test is presented in the phenomenon of hemolysis, which was first discovered and studied by Bordet and Gengou. This phenomenon consists of the well-known fact that if red blood cells of one animal are introduced into another of a different species, the blood of the latter acquires the power to dissolve the blood cells of the former when mixed with them in a test tube. The substances necessary for hemolysis are, (1) the hemolytic amboceptor, which is the serum of a rabbit that has been injected with washed sheep corpuscles; (2) the complement in the form of normal guinea-pig serum, and (3) washed blood corpuscles of the sheep. In the complement fixation test there are also used, besides the hemolytic system, the serum of the horse to be examined and an extract of glanders bacilli, termed antigen.

The complement fixation test is so called on account of the fact that the complement is fixed by the combination of glanders bacilli extract with antibodies in the serum of a glandered horse, and is thus prevented from participating in the hemolytic process in which it is essential in order for hemolysis to take place. By this method, even small quantities of glanders antibodies (amboceptors) can be demonstrated in a serum.

The complement fixation accordingly represents a specific test, as only in the presence of glanders antibodies and glanders antigen will a reaction take place. The results of the test should be interpreted as follows:

1. Horses in which the serum produces a complete fixation of the complement in quantities of 0.1 c.c. and 0.2 c.c. should be considered as glandered.

2. Horses in which the serum gives a complete fixation in the quantity of 0.2 c.c., and an incomplete fixation in the quantity of 0.1 c.c., should likewise be considered as glandered.

3. Horses in which the serum produces incomplete fixation in quantities of 0.1 c.c. and 0.2 c.c. should also be considered as glandered.

4. Horses in which the serum shows no fixation of the complement should be considered free from glanders.

In order to reduce the possibility of error to a mimimum, the agglutination test may be applied to the negative cases, and if this shows a value of 1 : 1,000 or over the animal should be considered as glandered. However, such cases are extremely rare.

The Effects of Subdural Injections of Leucocytes on the Development and Course of Experimental Tuberculous Meningitis: WILFRED H. MANWARING.

The injection of suspensions of tubercle bacilli into the basal meninges of dogs causes a tuberculous meningitis, characterized by a latent or incubation period of from five to thirty days, depending on the dosage and virulence of the culture injected, followed by a period of increasing paralysis and incoordination, ending almost invariably in death.

The injection of homologous leucocytes into the basal meninges of these animals, during the latent or incubation period of the disease, has the uniform effect of delaying the development of the paralytic symptoms. In dogs injected with small doses of tubercle bacilli of low virulence, the development of the paralytic symptoms has been prevented by this means for a period of seven months (up to the present time), while the untreated control animals, injected with the same doses, have all developed paralyses within a period of about four weeks, from which half of the untreated dogs have thus far died.

In press, *Journal of Experimental Medicine*, 1912.

Simple Methods in the Bacteriological Diagnosis of Cholera: CHARLES KRUMWIEDE.

Two points were kept in view, viz., the possibility of examining enormous numbers of cases with the minimum of equipment and the rapid preparation of media for immediate use in emergency.

In general, no medium is necessary but peptone water. If the feces contain a sufficient number of cholera vibrios, the peptone cultures after eight to twelve hours have practically a pure culture at their surface. If a drop of this and immune serum be mixed, the microscopic agglutination is so prompt and evident as to be diagnostic.

In examining carriers or mild cases the first peptone tubes may show little or nothing. If, however, some of the surface growth be subinoculated in a second series of peptone tubes and incubated, the surface growth becomes sufficiently pure for testing the agglutinability of the vibrios.

In four instances we have been unable to make a diagnosis till the end of the second enrichment.

Vibrios other than cholera can be excluded by their inability to enrich or by the absence of any influence of the agglutinating serum. Where they are few in number, as for instance in the first enrichment, a tentative diagnosis can be made by the influence of the serum on their motility. The motility of cholera and the very closely allied vibrios is so marked as to be evident even in mixed cultures.

We were able by this method to diagnose two carriers among the passengers of one ship, 50 per cent. of whom had cholera-like vibrios in their stools. The results were all verified by the use of the Dieudonne medium.

A Simple Selective Medium.—We have tried to avoid the use of defibrinated blood, which is not always obtainable in emergency. The following formula, substituting eggs, gives equally favorable results:

A. Whole egg and water a.a.
Sodium carbonate (crystalline, 12 to 13½ per cent.)
} Mix in equal parts, steam for 20 min.

B. Meat free agar, viz., peptone, salt and 3 per cent. agar.

Mix *A*, 30 parts, and *B*, 70 parts, while the agar is boiling hot. Pour medium to thick plates, allow them to stand open for 20 minutes to dry and then inoculate by surface streaking.

Should other fecal bacteria grow, the cholera colonies can easily be selected. The latter have a distinctive hazy outline and appear to be deep in the agar. With longer incubation a zone of clearing appears about the colonies.

Studies on Etiology of Equine Influenza: N. S. FERRY.

A Streptococcus, presumably the organism described by Schültz and others, has been cultivated uncontaminated from the trachea in the early stages of nearly every case of acute influenza studied.

An organism with the same characteristics has been isolated from the blood of thirty-four out of sixty-three cases.

Symbiotic relationship with the Staphylococcus, in broth cultures, seems to favor the growth of this Streptococcus.

This organism was able to pass many times through Berkefeld filters, and a few times through Chamberland filters, showing that it is a very minute organism in some stage of its life cycle.

The *Bacillus equisepticus* has not been seen in, nor isolated from, either the trachea or blood of a single case of influenza, therefore, Lingnieres's findings have not been corroborated.

We have not been able to find any points of difference between this organism and the Strepto-

coccus isolated from the abscesses in the cases of strangles.

Inoculation experiments were not extensive enough to warrant any conclusions as to the infectious nature of the organisms. We have record, however, of one case with the fever, prostration, cough and discharge from the nostrils, which was very characteristic of influenza. This followed the intravenous injection of a pure culture of the organism. We had another case of a large tumor at the site of inoculation after a subcutaneous injection of the same culture.

From the light of our work, up to the present time, we do not feel justified in stating positively that this Streptococcus is the cause of influenza in horses, but we do believe that the findings point very strongly to that conclusion.

If this proves to be the same organism that is found in strangles and also contagious pneumonia, and it is agreed by all authorities that the Streptococcus found in strangles is the cause of that disease, then, we believe, we are justified in putting forward the argument that this Streptococcus is the cause of the symptom complex we have termed "influenza," and that strangles and contagious pneumonia are not clinical entities, but complications of influenza due either to secondary infections or to extension of the primary disease.

The Influence of the Carrier in the Management of Institutional Diphtheria: J. J. KINYOUN.

The writer reports that since 1908 it has been the custom of the Health Department when a clinical case of diphtheria is reported from any institution to make an examination of all the contacts. Since beginning this there have been reported 23 clinical cases of diphtheria from 14 institutions. Cultures taken from all the contacts, immediate or remote, gave 220 carriers out of 2,004 contacts. In all the institutions save one there were no further cases. In one over which the Health Department acted only in an advisory capacity, and where there was not a complete culturing of all the contacts, other clinical cases did occur. Such cases did not cease until all the contacts were cultured. All bacillus carriers are considered as if they were clinical cases and are subjected to the same quarantine methods.

The writer finds that there is no arbitrary rule for the discharge of the carrier from quarantine, but must be done on the culture test. An analysis of the 199 cases of the carrier shows that the first negative culture from these was as follows: 20 cases on the 3d day; 18 on the 4th day; 30 on the 5th day; 34 on the 6th day; 30 on the 7th day; 22 on the 8th day; 10 on the 9th day; 2 on the 10th day; 5 on the 11th day; 10 on the 12th day; 9 on the 13th day; 3 on the 14th day; 3 on the 16th day; 2 on the 18th day; 1 on the 30th day. In none of these carrier cases were there any clinical symptoms.

The writer agrees with McDonald that the control of diphtheria is the control of the carrier.

A Panum Incubator with Important Modifications: LEO F. RETTGER.

In the construction of an incubator designed to meet the general needs of a bacteriological laboratory, the Panum model as described in Klöcker's "Fermentation Organisms" was chosen. The construction work was entrusted to a skilled coppersmith in New Haven. Copper was used throughout, except in the hinges of the doors, which are of brass, and the outer wall of the incubator, which was made of one-inch wood. Three inches of felt were packed between the outer and inner walls. Instead of being provided with four large outer doors which are fastened by hinges on the floor of the incubator, the incubator has eight doors, two for each main, square, compartment. The doors are in pairs, they swing on hinges and close in such a way that one door fits closely against the other. The doors are about three inches thick, and at the same time light in weight, as the space within the two walls is filled with air. Each of the eight compartments, excluding the refrigerator, is further provided with a glass door, which is easily removed. A gas safety lamp is the source of heat for the blood temperature end of the incubator. The compartment which is heated directly by the flame is surrounded completely with water. The water jacket is connected with a small water container which is made of copper. As the gas pressure is fairly uniform, this arrangement has given entire satisfaction. A Reichert thermo-regulator is installed.

When the refrigerator end is kept well supplied with ice the incubator is remarkably efficient. The temperatures in the different compartments are practically constant. This has been demonstrated particularly in a long series of experiments in which frequent and painstaking determinations were made.

All abstracts have been supplied by authors unless otherwise stated.

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